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PHYTOPATHOLOGY

VOLUME XIII NUMBER 9

SEPTEMBER, 1923

STUDIES ON THE PHYSIOLOGY OF SOME PLANT PATHOGENIC BACTERIA; VII. PECTIC FERMENTATION IN CULTURE MEDIA CONTAINING PECTIN

FREDERICK A. WOLF[1]

The thin central membrane or middle lamella of cell walls is constituted of a group of substances known as pectic substances. These pectic substances are found in practically all parts of green plants but are relatively most abundant in young rapidly growing parts, in fleshy roots, as beets, turnips, and artichokes, and in succulent fruits as apples, oranges, pears and currants. The common property of causing the gelatinization or jellying of fruit juices is in the popular mind, associated with pectic compounds, but they are of special interest to the plant pathologist by virtue of the fact that invading organisms can pass between host cells only after having attacked these substances, presumably by means of pectic enzymes.

It is to various biochemists that credit is due for a knowledge of the constitution of pectic substances and of their cleavage products. This phase of the problem together with an adequate historical treatment of the subject are comprehensively dealt with in a recent investigation by von Fellenberg (3). It should appear from the terminology of these biochemists that the name protopectin (pectose of older writers) is applied to the complex mother substance from which the simpler pectic compounds are derived. The protopectin of unripe fruits is insoluble but through the action of the enzyme pectosinase, during ripening, it is transformed into soluble pectin. Soluble pectin may then be coagulated to a gel by pectase, provided calcium or some other suitable base is present, and this gel then be hydrolyzed by pectinase, to reducing sugars or pectinase may hydrolyze pectin directly. According to von Fellenberg (3), the pectin complex is composed of mucic acid forming groups, methyl pentose, arabinose, carboxyl groups and methyl alcohol. The cleavage products could, therefore, be expected to serve well as a source of supply of carbon compounds for certain organisms.

[1] Previous publications in this series have been compiled in Technical Bulletin 20 of the North Carolina Agricultural Experiment Station.

Phytopathological research may be credited with having clearly shown, or at least strongly postulated, the possession by a number of fungi and bacteria, of the property of secreting pectic enzymes. The organism causing brown rot of stone fruits, *Sclerotinia cinerea*, may be cited as an example of the fungus which effects the decomposition of tissues by dissolution of the middle lamella and then assimilates the pectin in its own metabolism (4). Among the plant pathogenic bacteria, the soft rot of certain vegetables, *Bacillus carotovorus* (2) and the black rot of crucifers, *Bacterium campestre* (5) have no doubt been most thoroughly investigated as to their pectin relations.

It may be stated, without including an extensive review of literature on technique, which is not pertinent in this paper, that several methods have been used to demonstrate pectic enzyme activity by fungi and bacteria. In view of our limited knowledge of the quantitative activity of enzymes, all methods perforce are qualitative, some rather laborious, and none well adapted to the detection of weakly acting pectin ferments. Further, it does not appear that use has been made in any foregoing investigation of culture media containing pectin to determine this physiological property. A method for its preparation will, therefore, be described, data on its use recorded and a brief discussion accorded it.

Methods. A commercial product known as "Certo" prepared by the Pectin Sales Co., Rochester, N. Y., was used in the preparation of relatively pure pectin. The method employed was essentially like that used by Caldwell (1). To this Certo, an equal volume of 95 per cent alcohol was added which caused the precipitation of the pectin as a gelatinous fibrous rubber-like mass. The brown coloring matter of the Certo for the most part remains in the alcohol. This mass of pectin was collected on a cheesecloth in a porcelain filter, the alcohol worked out of it with a spatula and it was then washed with small quantities of fresh alcohol. To further purify the pectin, it was disolved in as small a quantity of warm water as would secure complete solution and re-precipitated with an equal volume of 95 per cent alcohol. The mass of pectin was again washed, pressed out and washed after which it was permitted to dry slowly at a temperature of 70° C. No coloring matter remains in the alcohol of the third precipitation. The dry pectin thus prepared is a whitish gray mass acidic in character which may be easily pulverized and dissolves readily in warm water.

Bacto-agar containing 18 grams to 100 cc of water or plain bouillon containing 1 per cent of Difco peptone, 0.3 per cent Liebig's beef extract and 0.5 per cent NaCl were then prepared as stock culture media. To these media, after they had been flasked, sterilized and cooled to

about 60° C., about 1 per cent by weight of powdered pectin was added which with agitation dissolved. The reaction was then adjusted to pH 7.4 by the addition of strong NaOH solution, and phenol red was introduced into the agar media as an indicator. Aseptic precautions were observed throughout, making unnecessary a final sterilization, which would have changed both the hydrion concentration (6) and the pectin content. The media were then poured into previously sterilized test tubes. After 48 hours' incubation, in order to insure their freedom from contamination, they were inoculated. The following organisms were employed: *Bacillus carotovorus*, *Bacterium tabacum*, *Bact. angulatum*, *Bact. sojae*, and *Bact. campestre*. In the liquid media, changes in reaction were followed by colorimetric readings at definite consecutive intervals.

Results. Cultures of these several organisms in pectin bouillon were first prepared, incubated at 25° C. and the changes induced by each recorded in the following table:

TABLE 1—*Changes in reaction in pectin bouillon, initial pH 7.4*

Organism	Age of cultures in days and pH concentration Days		
	3	5	7
	pH	pH	pH
Bacillus carotovorus	5.4	5.2	5.2
Bact. tabacum	7.0	6.8	6.4
Bact. angulatum	7.0	6.8	6.4
Bact. sojae	7.0	6.8	6.8
Bact. campesire	6.8	6.4	6.0

Manifestly all of these organisms are able to attack pectin with the formation of acid but of them the soft rot organism exhibits the most vigorous fermentative action. Since it has been shown in another study (6) that when cultured in plain bouillon all cause this medium to become progressively more alkaline except in the case of *Bacillus carotovorus*, in which an increase in hydrion concentration is followed by a reversal of reaction, it is apparent that this increase in acidity comes from the fermentation of pectin. In the light of the findings as to the constitution of the pectin complex, acid production can reasonably be regarded as postulating enzyme activity.

Pectin agar containing phenol red as an indicator was employed in order to check these changes in reaction and provide an ocular demonstration of this progressive change. In this medium, the color had all disappeared, in stab cultures of *Bacillus carotovorus*, within 3 days whereas there was only a slight change beneath the surface of the agar

in the case of the other organisms. By the seventh day, the color had disappeared in stab cultures of cabbage black rot, very pronounced change had taken place in the cultures of the tobacco leaf disease organisms, but only slight change in the case of the soybean blight. The rate of the disappearance of color is thus in accord with the findings in liquid cultures.

Discussion. In the case of organisms which vigorously attack the middle lamella of succulent tissues, as is the case with *Bacillus carotovorus* as demonstrated by Jones (2) and *Bact. campestre* as demonstrated by Smith (5), the solvent action of pectic enzymes is easily demonstrated by any one of several methods. For such, the development of additional methods for the qualitative determination of pectic enzymes may have no special value. For organisms of the type of the others, whose pectin relations are probably like those of the great majority of leafspot disease producers, it is either impossible or difficultly possible, because of their relatively weak pectin decomposition, to demonstrate pectic enzymes. For such, this method is believed to be adapted because of its delicacy since a slight increase in acidity can be both easily and readily measured. It should also facilitate a study of the invasion of host tissues by this type of organism and of the pathological anatomy.

Summary. Pectic fermentation may be postulated in culture on media containing pectin. Increase in acidity is made the criterion on which the presence of pectic enzymes is shown.

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DAMPING-OFF OF TAPROOTS OF CONIFERS

ANNIE E. RATHBUN

Formerly the term damping-off was used to designate primarily the death of a seedling which resulted from the attack of a parasitic fungus at the soil surface; this caused there a local constriction of the stem and later the fall and wilting of the seedling. However, several years ago Büttner (1) recognized that root infections were frequent, while Hartley (2, 3) in his description of normal damping-off stated that in sandy soils root infections are more common than hypocotyl infection and that damping-off in nursery beds is in most cases, primarily a root rot of some type. These conclusions have been based for the most part upon either field observations or artificial infections of coniferous roots produced indirectly by the same methods of soil inoculation employed in most other damping-off and root rot investigations (2, 3). This indirect method does not show for normal damping-off the amount of injury which results respectively from root and from hypocotyl infections; and it does not indicate the relative degree of parasitism of the different fungus species when free from fungous inhibitors and soil. In order to secure some information on these points several small direct inoculation experiments with the taproots of seedlings of *Pinus banksiana* Lamb., *Pinus resinosa* Ait. and *Picea engelmanni* Engelm., have been conducted by the writer. Inoculation was made while the seed coats were still clinging to the cotyledons of the seedlings. The seedlings were grown in sand which had been autoclaved and watered with boiled water. Three very similar methods of inoculation were used (4).

The first method was to cut away one-half to two-thirds of a Petri dish culture; then to place the seedlings in the Petri dish in such a way that the roots were in contact with the inoculum while the stems touched only the glass. Both corn meal and prune agars were used.

A second method was to grow the fungi on rice mush in Erlenmeyer flasks. Several grains of the rice plus the spores and mycelium growing upon them were transferred to the bottom of a sterile Petri dish. Then the seedlings were laid in the Petri dish with the root of each seedling in contact with the inoculum at one point. With this method the seedlings showed a slight tendency to wilt.

In the third method in order to overcome this wilting tendency the bottom of each Petri dish was lined with filter paper moistened in boiled water before the addition of the inoculum. Otherwise this method did not differ from the second.

Control seedlings were treated similarly to the inoculated ones except that sterile rice or agar from the same batch as the inoculated medium, was used. Each control and each inoculated unit consisted of ten seedlings. The duration of each experiment was four days.

Before removing the seedlings from the sand each pot was thoroughly soaked in boiled water. This allowed the seedlings to be removed from the sand more easily and consequently reduced the possibility of injury to the root hairs or small rootlets thus checking much of the physiological disturbance which otherwise might have resulted.

The inoculating fungi in these experiments were *Pythium debaryanum* Hesse, *Corticium vagum* B. and C., Fusarium spp., Botrytis spp. and certain miscellaneous fungi.[1] Many of them have been tested by Hartley (2, 3) in various indirect inoculation experiments and all of them have been used by the writer in the direct inoculation of coniferous seeds, young radicles, and stems. The results secured from inoculating these last three will be published elsewhere.

DISCUSSION OF RESULTS

The results of the present investigation are given in table 1. On account of the small number of experiments and the failure to secure distinctly different results from the different methods, it was thought best to present merely a summary of the results instead of comparing the relative virulence of the various strains on the different media. The fact that the controls of *Pinus banksiana* and *P. resinosa* and in all but one experiment, those of *Picea engelmanni* always remained free from rot indicates that the rots observed were really parasitic effects and not consequent upon the removal of the seedlings from the soil.

Pythium Debaryanum

Pythium debaryanum is very parasitic to the taproots of *Pinus banksiana*, *P. resinosa* and *Picea engelmanni*. There is apparently, so far as these experiments show, no significant difference in the susceptibility of the three species. The tested strains seem to exhibit approximately equal virulence whether they were isolated from coniferous or other hosts. Moreover, except for one case with each of two strains they were always of approximately equal high virulence. This seems to indicate that some other factor, such as inability to maintain themselves in the soil until the seeds had germinated, contributed to the difference in the virulence of these same strains reported by Hartley (2, 3). It is very possible if a larger number of strains had been tested in these

experiments, a greater range in virulence variability would have been secured.

Corticium Vagum (*Rhizoctonia solani* Kühn).

Many of the *Corticium vagum* strains were used in only one experiment while others showed great variability in their ability to attack coniferous taproots. Two strains were consistently highly virulent and two others were consistently non-parasitic to taproots. On the whole, *Pinus resinosa* seemed more resistant than the other two hosts to this fungus. However, further experimentation is necessary to establish this point. The reason for the apparently greater resistance of *Pinus resinosa* may be the fact that fewer Corticium strains were used on it than on either of the other hosts. As far as these experiments show the strains isolated from coniferous hosts were not significantly more parasitic to taproots of conifers than the strains isolated from other hosts.

Fusarium spp.

The single tested strains of *Fusarium sporotrichioides* Sherb., and *F. arthrosporioides* Sherb., apparently equalled the most virulent strains of *Pythium debaryanum* in parasitism to taproots. *Gibberella saubinetii*? (Mont.) Sacc. and species of the moniliform section of Fusarium were moderately parasitic. There is no evidence to prove that the strains of the latter isolated from coniferous hosts were more parasitic than those isolated from other hosts. The other species of Fusarium were either doubtfully parasitic or non-parasitic. There is some indication, which needs confirmation, that *Picea engelmanni* is less resistant than the pines to some of the Fusarium species.

Miscellaneous Fungi

Rheosporangium aphanidermatus Edson equalled *Pythium debaryanum* in ability to attack taproots. The large sclerotial Botrytis, commonly called *B. cinerea* Pers., was decidedly parasitic to the taproots of *Pinus banksiana*, but gave less evidence of virulence when used on *Pinus resinosa*. *Phomopsis juniperovora* Hahn was able to attack the taproots of *Picea engelmanni* and *Pinus resinosa* while Phytophthora sp., was able to attack the latter. *Rhizoctonia potomacensis* Wr., which may be only a strain of *Corticium vagum*, gave some slight evidence of parasitic ability while *Pythium artotrogus* (Mont.) de Bary and *Mucor racemosus* Fres. showed no evidence of parasitism.

TABLE 1—*Results secured from the inoculation of taproots of Pinus banksiana, Pinus resinosa and Picea engelmanni with various fungi*

Inoculating fungus**	Pinus banksiana				Pinus resinosa				Picea engelmanni			
	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed
Rheosporangium aphanidermatus	1	10	10	100	1	10	10	100	—	—	—	—
Fusarium arthrosporioides (a)	1	20	20	100	—	—	—	—	1	30	29	97
Pythium debaryanum												
All strains	10	260	255	98	10	170	169	99	8	160	150	94
From coniferous hosts	5	110	110	100	5	70	69	99	4	50	40	80
From other hosts	5	150	145	97	5	100	100	100	4	110	110	100
Botrytis cinerea												
All strains	4	40	34	85	6	70	19	27	1	20	0	0
From coniferous hosts	—	—	—	—	1	20	5	25	—	—	—	—
From other hosts	4	40	34	85	5	50	14	28	1	20	0	0
Corticium vagum												
All strains	18	320	180	56	7	90	18	20	13	260	139	53
From coniferous hosts	14	240	157	66	5	70	13	19	9	180	106	59
From other hosts	4	80	23	29	2	20	5	25	4	80	33	41
Rhizoctonia potomacensis (c)	1	20	10	50	—	—	—	—	1	10	4	40
Gibberella saubinetii ? (a)	1	40	16	40	1	20	0	0	1	40	34	85
Fusarium martii Ap. and Wr. (a)	1	10	4	40	—	—	—	—	1	10	6	60
Fusarium moniliforme group (a, b)												
All strains	11	150	37	25	4	40	9	23	10	170	79	47
From coniferous hosts	7	90	25	28	1	10	5	50	7	100	41	41
From other hosts	4	60	12	20	3	30	4	13	3	70	38	54

From other hosts	4	60	12	20	3	30	4	13	3	70	38	54

Inoculating fungus**	Pinus banksiana				Pinus resinosa				Picea engelmanni			
	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed	Number of strains	Number of seedlings inoculated	Number of seedlings decayed	Percent of seedlings decayed
Phythium artotrogus	1	10	1	10	1	10	1	10	—	—	—	—
Fusarium culmorum group (a, b)	3	30	0	0	—	—	—	—	3	30	0	0
Fusarium elegans group (a, b)	1	10	0	0	5	50	5	10	1	10	10	100
Fusarium solani (Mart) Ap. and Wr.	1	10	0	0	—	—	—	—	1	10	9	90
Fusarium between discolor and roseum groups (b)	1	10	0	0	1	10	2	20	—	—	—	—
Fusarium sporotrichioides (a)	—	—	—	—	—	—	—	—	1	20	20	100
Phomopsis juniperovora	—	—	—	—	2	20	15	75	1	20	14	70
Phytophthora sp.	—	—	—	—	3	30	14	47	—	—	—	—
Fusarium hyperoxysporum Wr.	—	—	—	—	1	10	3	30	—	—	—	—
Fusarium vasinfectum Atk.	—	—	—	—	1	10	0	0	—	—	—	—
Fusarium ventricosum Ap. and Wr.	—	—	—	—	1	10	0	0	—	—	—	—
Mucor racemosus	—	—	—	—	1	10	0	0	—	—	—	—
Controls	—	400	0	0	—	175	0	0	—	350	30	9*

* Three control plates infected in one experiment which was badly overgrown with *Mucor sp.*

** Fungi arranged in order of apparently decreasing virulence to *Pinus banksiana.*

(a) Identified by Miss Helen Johann.

(b) Identified by Dr. C. B. Sherbakoff.

(c) May be a strain of *Corticium vagum* according to H. A. Edson who furnished the culture.

The fact that with the exception of *Fusarium arthrosporioides*, no fungus which has not previously been reported (2, 3, 5) as a potential damping-off parasite caused in these investigations serious decay of taproots, seems to indicate that these methods of inoculation really do show which species are likely to be most virulent in the field. However, it apparently shows that some strains especially of *Pythium debaryanum* were formerly considered slightly or non-parasitic because of their inability to maintain themselves in the soil or to combat fungous inhibitors until the seeds had germinated. Even these less virulent strains would possibly do considerable damage in nursery beds if they happened to infect the soil at a time when the taproots were young. *Pythium debaryanum*, *Corticium vagum*, *Fusarium sporotrichioides*, *Botrytis cinera* and *Phomopsis juniperovora* have previously (5) been reported as causing, under artificial conditions, more or less serious root rot of pine seedlings too old to succumb to the more ordinary types of damping-off. The same species have been found virulent on young stems and radicles, while both *Mucor racemosus* and *Pythium artotrogus* have been found to be non-parasitic. Further experimentation is necessary to definitely establish the relative susceptibility of the taproots of the above hosts as well as the relative virulence of the tested fungi.

SUMMARY

1. These direct inoculations of taproots of very young coniferous seedlings in Petri dishes seem to indicate that differential results can be secured by the use of these methods.

2. *Pythium debaryanum*, *Rheosporangium aphanidermatus*, *Fusarium arthrosporioides* and *F. sporotrichioides* are very virulent, under the artificial conditions of these experiments, to the taproots of young conifers. Since the last three, despite the large amount of diagnostic work recently reported (3), have never been found naturally infecting coniferous seedlings, *P. debaryanum* is considered the most dangerous parasite of coniferous taproots.

3. *Corticium vagum*, *Botrytis cinera*, *Phomopsis juniperovora*, Phytophthora sp., and strains of the moniliform section of Fusarium are apparently able to cause considerably decay of coniferous taproots.

4. *Mucor racemosus*, *Pythium artotrogus* and certain Fusarium species are apparently non-parasitic to coniferous taproots.

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The cultures used in these experiments belonged to Dr. Carl Hartley. He obtained them from D. Atanasoff, M. F. Barrus, H. A. Edson, L. L. Harter, L. A. Hawkins, G. N. Hoffer, Mrs. C. R. Tillotson, H. H. Whetzel and various members of the Office of Forest Pathology. Miss Helen Johann identified Mr. Atanasoff's culture and Dr. C. B. Sherbakoff named several of the other Fusarium cultures. The writer wishes to express her thanks to the above people for their kind assistance and especially to Dr. Hartley for valuable suggestions.

THE RELATION OF SOIL MOISTURE TO FORMALDEHYDE INJURY OF ONION SEEDLINGS

P. J. Anderson

With two Figures in the Text

In controlling onion smut by the application of formaldehyde in the furrow, only fairly dilute solutions may be used. If concentrated solutions are applied, even though the actual quantity of formaldehyde per unit of row is no greater, the seeds fail to germinate. Almost from the beginning of the practice of controlling smut in this way, it has been customary to dilute the solution by adding 1 pint of the commercial formaldehyde (said to be a 40 per cent solution of the gas in water, but usually analyzing less) to 16 gallons of water, thus making a solution containing 1 part of formaldehyde to 128 parts of water. The standard recommendation now is to apply this dilute solution at the rate of 16 gallons to about 3000 feet of drill. For convenience in discussion and for ready comparison with other rates of dilution and application, this standard may be called "the 1-128–3000 formula." Although smut can be controlled by the use of this formula, the practical onion grower finds certain serious objections to the use of so much water. It makes the soil so wet that the seed sprout, packing wheel or coverers frequently become clogged with mud. A more serious objection is the extra labor involved in drawing so much water, in more frequent refilling of the tank and particularly the increased weight of the drill when so much water must be pushed across the field. Inquiry among the growers of the Connecticut Valley onion region brought to light the fact that, although the dilution of 1 part to 128 had been recommended by the experiment stations for many years, none of them were using it. They preferred to use a smaller quantity of a more concentrated solution even at the risk of killing the seed. Recognizing the futility of recommending any longer a formula which the practical grower would not use, the Department of Botany of the Massachusetts Agricultural Experiment Station started investigations in 1918 with the object of determining whether the extreme dilution of the 1-128–3000 formula was necessary, and if not, to find what formulas involving less dilution could be safely used. The results of the investigation as regards the control secured by use of formulas involving more concentrated solutions will be presented in a future publication: the present paper will be devoted to a discussion of some phases of formaldehyde injury.

During the first years of the field investigation it was found that concentrated formalus such as 1-64–3200 and 1-50–3000 could be safely used with but slight apparent reduction in the percentage of onions which came up. When, however, in 1921, the same formulas were used on the experimental fields, they caused very decided loss in the number of seed which sprouted as compared with the adjacent untreated rows. Practically all of the formulas tried caused more or less injury. Many growers sustained so much loss from the use of formaldehyde that they plowed up the fields and planted other crops. We were at first inclined to believe the trouble was due to a difference in the character of the formaldehyde which was used. Samples of formaldehyde were therefore collected by A. V. Osmun and the writer from sixteen different sources, including among them samples of the same material which we had used during the previous years and which had not caused injury. Chemical analyses of the samples revealed no differences in composition which were of sufficient size or character to warrant a suspicion of their toxic effect. The manufacturers assured us that there had been no change in the method of manufacture of formaldehyde. The most apparent difference was in the percentage of methyl aclohol contained in the various samples. The sixteen samples were next tested simultaneously on onion seeds sown in greenhouse benches, using for each the formulas 1-50–3000, 1-50–5000, 1–100–3000 and leaving untreated rows between the plots. In order to see what effect variation in the percentage of methyl alcohol would have, samples containing known percentages from 4 per cent to 16 per cent alcohol were included in the tests. Without discussing the results in detail, it may be briefly stated that(1) the source of the formaldehyde was without influence on the percentage of plants which came up, (2) variation in the percentage of methyl alcohol made no difference, (3) with all samples and every formula used, formaldehyde *retarded* germination for a day or two and, (4) with all samples and formulas, formaldehyde *prevented* the germination of a certain percentage of the seed, this loss being more apparent when a more concentrated solution was used or where the quantity of the solution was increased. The results of these experiments eliminated the possibility that the losses of 1921 were referable to difference in the character of the formaldehyde.

Since there had been no differences in the formulas or method of application of formaldehyde nor in the treatment of the soil in 1921, there appeared to remain only the character of the season itself as a possible explanation of the trouble. The character of the spring weather could obviously affect the soil—where the injury occurs—in two ways:

(1) in the percentage of moisture and (2) in the temperature. The experiments described below were undertaken with the object of determining to what extent the percentage of injury is influenced by variation in the percentage of moisture in the soil. The effect of the soil temperature on injury has not been determined as yet.

EXPERIMENT WITH SOIL A.

Soil A was a typical sandy onion soil of the Connecicut Valley. The moisture retentive capacity of this soil was found to be 51 per cent of the dry weight of the soil.[1] The experiments with Soil A were conducted during the winter months in the greenhouse in 8-quart glazed pots without drainage openings in the bottom. About an inch of small stones was placed in the bottom of each pot, a weighed amount of sifted soil added and brought up to the desired moisture content by pouring water from above. After settling for a day the soil was worked up thoroughly and 100 seeds planted in a circular row in each pot. The desired quantity of formaldehyde solution was dripped over the seed from a burette and immediately covered. As a check for each treated pot a second pot received the same treatment except that water instead of the formaldehyde solution was dripped over the seed. All pots were brought up to the original weight by addition of water every second day, alternating between surface sprinkling and subwatering through a deep hole in the center of the soil. The percentage of germination was counted at the end of 2 weeks, 3 weeks, and 4 weeks. The results of the two sets of experiments with this soil are tabulated below. (Tables 1 and 2.)

This preliminary experiment indicated that the soil with 11 per cent moisture was too dry for good germination, while that with 40 per cent was too muddy. Leaving these two out of consideration, it was evident from this experiment that the formaldehyde injury was greater in the dry soils and decreased as the percentage of soil moisture increased.

In the next experiment the extremely dry and wet pots were omitted and the intervals in moisture content between pots was diminished. The results of the second experiment are presented in table 2 and represented graphically in figure 1.

[1] The retentive capacity was determined as follows: Two samples of the soil were dried in the electric even at 105 C. for 24 hours, then sifted through a 1 mm. sieve. One hundred grains of each sample were then placed in celluloid cylinders of 1 inch diameter supported upright on a ring stand and closed at the bottom with filter paper disks held in place by pieces of fine cheesecloth over the bottom. Water was poured into the top of the cylinders until it saturated the soil and dripped from below. After the drip had ceased the soil was found to weigh 151 gms. The same method was used for determining the moisture retentive capacity of all soils used in subsequent experiments.

TABLE 1—*Results of formaldehyde treatment of onion seeds with different percentages of soil moisture*

Percentage of soil moisture	Plants up after 2 weeks			3 weeks			4 weeks		
	Treated	Check	Percent Loss	Treated	Check	Percent Loss	Treated	Check	Percent Loss
11	0	0	0	0	21	100	0	25	100
17	12	84	86	33	84	61	33	86	61
23	10	80	87	35	84	58	40	84	53
28	37	70	47	54	72	25	54	72	25
33	33	64	49	47	68	31	48	68	29
40	19	64	70	37	74	50	38	74	49

This experiment shows more clearly than Experiment 1 that the greatest injury is in the dry soil and that the percentage of injury decreases fairly regularly with the increase in the percentage of soil moisture. Figure 1 also illustrates very clearly the retarding effect of the formaldehyde: the difference between the treated and untreated rows at the end of two weeks (represented by the uppermost broken line) was considerably greater than at the end of three or four weeks. This same retarding effect was noted and measured in all the later experiments, but is not indicated in the tables presented below.

EXPERIMENTS WITH SOIL B.

Soil B had a higher percentage of organic matter and less sand than soil A, therefore a higher moisture retentive capacity. By the method previously described the retentive capacity was found to be 74 per cent. All the succeeding experiments were conducted with soil B. For these experiments, regular galvanized iron soil pots of 30 inch diameter and 14 inch depth were used. Two $\frac{5}{8}$ inch galvanized pipes projected above the soil on opposite sides of pots and were connected below by a perforated half-cylinder arm. Water was added on alternate days by pouring down the side pipes and by sprinkling from above. All pots were kept up to weight throughout these experiments as in the previous ones.

Purposes of the experiments with soil B. By the previous experiments it has been determined that formaldehyde injury varies inversely as

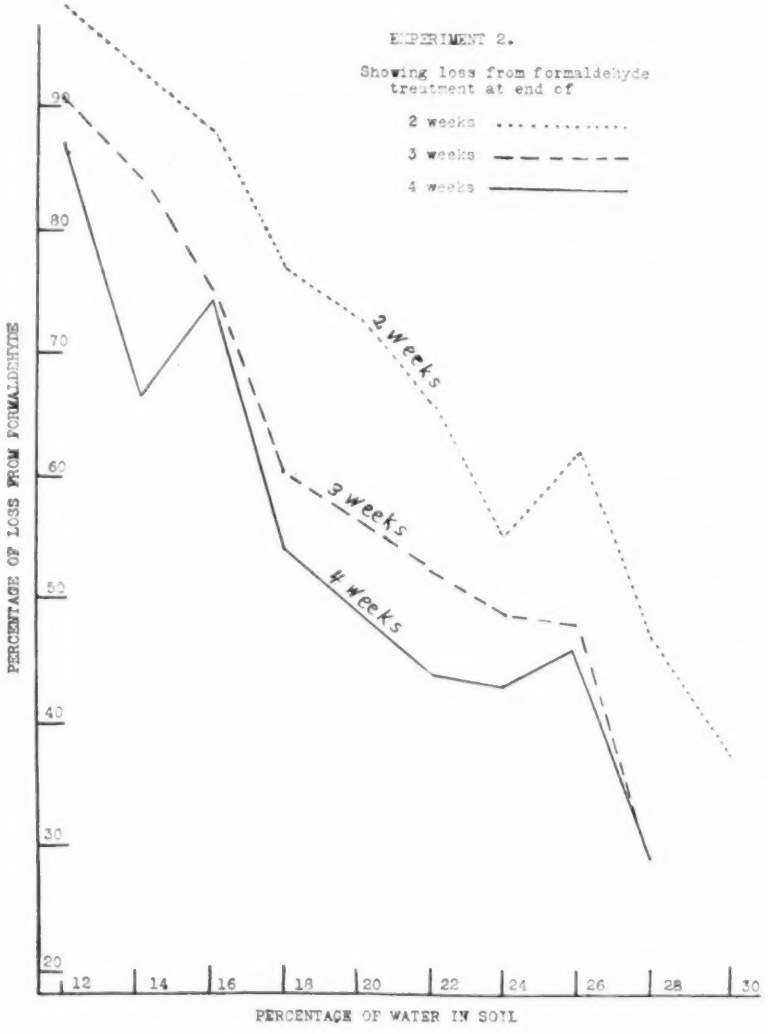

FIG. 1. Graph, showing the decrease in injury with increase in soil moisture.

the percentage of soil moisture when the 1-50–3000 formula is used. It was next desired to find out what the effect would be (1) when the formaldehyde is applied in more dilute solution and (2) when a smaller

TABLE 2—*Results of treatment of onion seed with formaldehyde (Formula 1-50-3000) with different percentages of soil moisture*

Percentage of soil moisture	Plants up after 2 weeks			3 weeks			4 weeks		
	Treated	Check	Percent Loss	Treated	Check	Percent Loss	Treated	Check	Percent Loss
12	1	66	98	6	68	91	10	69	87
14	5	81	93	14	76	84	25	76	67
16	9	79	88	20	80	75	20	80	75
18	19	83	77	34	81	60	37	81	54
20	21	77	73	35	76	54	39	76	49
22	26	76	66	36	77	53	43	77	44
24	34	76	55	40	78	49	44	78	43
26	30	80	62	42	82	48	43	82	47
28	38	72	47	52	71	29	52	71	29
30	49	77	37	55	77	28	55	77	28

quantity of the 1-50 solution per unit of row is applied. To test the first, the standard 1–128–3000 formula was used and for the second, the 1-50–4000 and 1-50–5000[1] formulas were tried. A third object was to see whether a change in the character of the soil would affect the results.

Effect of percentage of soil moisture on germination of the seed. In soil A it was found that the percentage of seed germination fell off rapidly below a moisture content of 12 per cent and above 30 per cent. Germination within the 12–30 limit was fairly constant and was regarded as normal. In soil B, with its higher retentive capacity, the range of normal germination was considerably higher. Considering as normal only those pots in which the seeds germinated to more than 60 per cent

[1] This formula represents approximately the rate of application used by many growers in the Connecticut Valley onion region. They add 1 gallon of formaldehyde to 1 barrel (50 gal.) of water and distribute this amount over 1 acre of onions. This formula is very convenient in practice and probably owes some of its popularity to that fact.

it will be observed from the data in the second column[1] of table 3 that the normal range extended from 24 to 44 per cent. The germination below and above these figures was erratic and uncertain. Their value

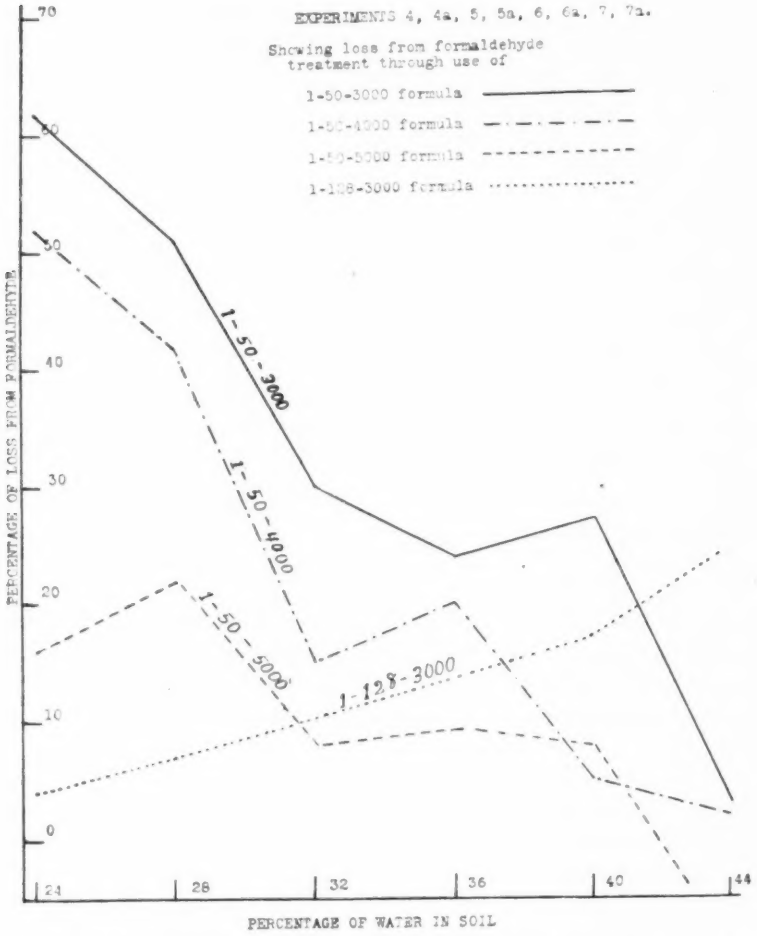

FIG. 2. Graph, showing effect of diluting the formaldehyde solution and of planting when the soil water is low.

for comparison is therefore doubtful. In considering the lack of germination in the treated very dry or very wet pots, there was always

[1] Each figure in this column is the average germination of eight check pots (800 seeds) for this particular soil moisture in the 8 experiments conducted with Soil B.

TABLE 3—*Summary of Experiments 4, 4a, 5, 5a, 6, 6a, 7, 7a.*

Percentage water in the Soil	Av. Germination of 8 checks	Exp. 4 + 4a 1-128–3000			Exp. 5 + 5a 1-50–3000			Exp. 6 + 6a 1-50–4000			Exp. 7 + 7a 1-50–5000		
		Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss	Av. Germ.	Dif. from av. ck.	Percent loss
16	8	0	8	100	0	8	100	—	—	—	—	—	—
20	28	27	1	4	9	19	61	6	2	25	9	19	67
24	69	66	3	4	26	43	62	33	36	52	58	11	16
28	79	73	6	7	39	40	51	46	33	42	62	17	22
32	71	64	7	10	50	21	30	60	11	15	65	6	8
36	74	64	10	13	52	18	24	59	15	20	67	7	9
40	76	63	13	17	55	21	27	72	4	5	70	6	8
44	68	51	17	25	67	1	2	65	3	3	75	—7	—10
48	51	28	23	45	58	—7	—13	55	—4	—8	56	—5	—9
52	41	8	33	80	50	—9	—22	34	7	17	30	11	27
56	17	5	12	70	42	—25	—147	3	14	83	6	11	65

in the writer's mind a question as to whether the injury was due to the condition of the soil or to the formaldehyde treatment. Although the data for all moisture percentages which were tried is presented in table 3, it is probably safest to use the results of only those between 24 and 44 per cent in drawing conclusions.

Methods for experiments 4, 4a, 5, 5a, 6, 6a, 7, 7a. The four formulas 1-128–3000, 1-50–3000, 1-50–4000 and 1-50–5000, were tested in soil ranging at intervals of 4 per cent from 16 to 56 per cent. For each treated pot there was one check pot of the same moisture content and treated in the same way throughout the experiment except that water instead of formaldehyde was applied to the seed when planted. Each experiment was repeated and each of the figures in table 3 represents the average of duplicate experiments. Percentages of germination were recorded at the end of 2 weeks, 3 weeks and 4 weeks as in the previous experiments, but only the results at the end of 4 weeks are given in table 3. The percentage of loss in each case is computed from the difference between the average germination of the two treated pots and the average of the 8 check pots. It is believed that the average of the 8 gives a more accurate basis of comparison than merely the two which were used for that particular experiment.

Discussion of results. (1) As will be seen by inspection of table 3 and more readily perhaps in the graph (Fig. 2) the results of the experiments with the 1-50–3000 formula confirm our previous conclusion that the amount of injury varies inversely as the moisture content of the soil. The maximum injury was not as high as for the experiments with soil A. This may have been due to a difference in the character of the two soils, but additional experiments are necessary before any certain conclusions on this matter can be drawn. (2) When the amount of the 1-50 solution per unit of row is reduced, as in the 1-50–4000 and the 1-50–5000 formulas, the amount of injury in the dry soils is reduced proportionately. (The effectiveness of the solution for the control of smut is probably reduced in the same ratio.) (3) When the actual amount of formaldehyde per unit of row is kept constant, but the dilution is increased, the injury in dry soils is diminished. Thus with the 1-128–3000 formula the injury in a dry soil is no greater than in more moist soils; in fact the results of experiments 4 and 4a seem to indicate that there is *less* injury in dry soils. This apparent increase in injury with increase in soil moisture may be partly explained in this case by the deleterious effect of the addition of so much water to the more moist soils at the time of planting the seed. This was apparent when these pots were compared with their own checks instead of with

the average of the eight. As far as effective control is concerned these results vindicate the standard 1-128–3000 formula as the safest one to use.

TIME OF THE INJURY

At what time after the seed is planted is the injury produced? This question is of considerable practical importance as will be indicated below. There would seem to be three possible answers: (1) The seed may be injured or killed immediately or within a few hours after planting by coming in contact with a fairly concentrated solution. (2) Injury may result from the repose of the seed for several days in a formaldehyde-soaked soil. (3) The injury may be due to the action of the formaldehyde on the young radicle and cotyledon when they emerge from the seed three or more days after planting. If the first of these three possibilities is the true explanation, then the amount of injury may be predicted from the condition of the soil on the day when the seed is planted, i. e., the weather conditions immediately *preceding* the planting day will determine the amount of injury. If, on the other hand, either of the other two is the true explanation, the percentage of injury may be greatly influenced by weather conditions *subsequent* to the day of planting; e. g., a heavy rain occurring the next day after planting might dilute the formaldehyde to such an extent that injury would be greatly reduced or prevented.

In order to determine this point the following experiment was conducted: Six rows of onions were planted in a greenhouse bench in the usual way. Half of them were treated with formaldehyde according to the 1-50–3000 formula, the other half being left untreated. At the same time on each succeeding day, 100 seeds from the treated rows and 100 seeds from the untreated rows were dug out, washed for an hour in running water to remove formaldehyde and then planted in another part of the bed in new soil. The ground during the experiment was fairly dry, but the percentage of water was not determined. Four weeks after the experiment was started the seedlings which had come up were counted. Table 4 below shows the results:

The average difference in viability between the treated and untreated seeds is 13. The difference was not less when the transplanting was done one day after treatment. There is no evidence here of an increase in injury with the length of time during which the seed remained in the formaldehyde-soaked soil. This experiment indicates then that the injury is caused directly to the seed on the day of treatment.

TABLE 4—*Experiment 3; showing the number of treated and untreated seeds which germinated after washing and transplanting*

Days between planting and digging	Treated	Check	Difference
1	60	76	16
2	49	52	3
3	65	79	14
5	55	73	18
6	58	81	23
7	73	77	4
8	63	75	12

PRACTICAL APPLICATION OF THE RESULTS

When the soil is very dry it is not safe for the grower to use as concentrated a formula as 1-50–3000. If, however, the soil is fairly wet on the planting day, he may save considerable labor and time by using a concentrated formula and at the same time he will sustain no more, or at least little more, loss of seed than if he used a dilute formula like 1-128–3000. With some practice he could probably learn to guage his formula of application according to the condition of the soil on that day. It is not likely that the weather of succeeding days will influence the results. If the soil is extremely dry and dusty, it is safest to use a dilution approximating the 1-128–3000 formula. Since there is always some loss from the use of formaldehyde, the grower should plan to use more seed per acre than on fields where smut is not present. Some growers have told the writer that they have practised this in past years.

SUMMARY

1. Any application of formaldehyde which is strong enough to control smut also causes a certain percentage of the seed to fail to germinate.

2. When a concentrated formula like 1-50–3000 is used, the amount of injury depends on the moisture condition of the soil. Injury varies inversely as the percentage of moisture.

3. The amount of injury may be reduced by diminishing the amount of the solution applied per unit of row, but the percentage of smut control is reduced in the same ratio.

4. The amount of injury in a dry soil may be reduced by increasing the dilution of the formaldehyde without at the same time reducing the actual amount of formaldehyde per unit of row.

5. The amount of injury can be predicted from the moisture condition of the soil on the day of planting. It is not affected by weather conditions during the subsequent days.

6. The grower could save time and labor by changing his formula according to the soil condition at the time of planting.

7. More seed should be applied when formaldehyde is used.

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SEVERITY OF ATTACKS OF BLACK STEM-RUST IN DENMARK, 1884–1921

HANS R. HANSEN

Many observations made in Denmark by growers for more than a century show that the interplay existing between black stem-rust (*Puccinia graminis*) and barberry is the cause of the widespread and destructive attacks of the former. Gradually as more and more evidence of the guilt of the barberry was produced, the campaign for and against the eradication of that plant waged hotter and hotter. Even after scientific tests had confirmed the evidence of the practical man, the fray continued and was fiercest among the farmers themselves.

In 1869 an attempt was made to exterminate the barberry by law, a paragraph in the Enclosure Act of March 6. However, it is certain that this paragraph was seldom put into effect, for barberry bushes continue to exist in great numbers and the black stem-rust to destroy. By untiring efforts for many years, Professor E. Rostrup makes clear the importance of exterminating the barberry. He collects evidence of the attacks of black stem-rust co-existing with the barberry bush in various sections of Denmark and publishes the facts in annual reports from 1884. During the nineties Rostrup estimates that the damage caused by stem-rust amounts to about 10 million kroner annually. At length he succeeds in promulgating a measure forbidding all cultivation of the barberry bush in Denmark outside of botanical gardens. On March 27, 1903, the measure becomes a law which goes into effect in 1904. At present the barberry bush may be said to have totally disappeared, even though a stray bush or two may be occasionally found in gardens. Simultaneously with the disappearance of the barberry bush the severe attacks of rust have also ceased.

The annual reports on plant diseases issued first in 1884 by Professor Rostrup, later continued by Professor Kolpin Ravn and now by the Phytopathological Experiment Station give a summary of the severity of the attacks of each year. According to these reports the attacks may be grouped into 3 classes: great damage in large sections of the country, sporadic appearance without importance, and very seldom found. In the second column of the table the severity of the attacks in the years 1884–1921 is shown.

TABLE 1—*Severity of attacks of black stem-rust and climatic conditions of the summer*

Year	Severity of attacks	Deviation from average precipitation in pCt	Deviation from average temperature C°	Deviation from average cloudiness (0—10)
1884	⊙	÷ 88	+0,9	÷1,7
1885	⊙	÷ 62	÷1,6	÷0,5
1886	⊙	÷ 90	÷1,6	÷0,7
1887	⊕	÷140	0,0	+1,2
1888	⊙	+120	÷4,2	+3,1
1889	⊕	+ **24**	**+2,8**	÷1,4
1890	⊙	+ 91	÷2,6	+2,6
1891	⊙	+122	÷1,1	+1,7
1892	ℂ	+ 39	÷2,9	+0,6
1893	ℂ	÷ 42	+1,9	0,0
1894	⊕	+ **37**	**+0,3**	**+1,3**
1895	⊕	+ **66**	÷0,3	**+1,9**
1896	⊕	÷ 49	**+3,4**	**+0,2**
1897	⊕	+ **24**	**+3,4**	**+0,9**
1898	⊙	+ 87	÷3,0	+2,2
1899	ℂ	÷167	+1,7	÷1,9
1900	⊙	+ 25	+1,8	+0,1
1901	⊕	+ **10**	**+3,2**	÷0,1
1902	ℂ	+ 4	÷5,0	+2,5
1903	⊙	+ **51**	÷2,1	**+2,6**
		Barberry Law passed 1903		
1904	⊙	÷113	÷0,6	÷0,9
1905	ℂ	+ **35**	**+2,7**	**+1,0**
1906	ℂ	÷ 40	+1,0	+0,4
1907	ℂ	+ 84	÷5,3	+4,6
1908	ℂ	÷ 9	+0,6	+0,6
1909	⊙	÷ 2	÷3,7	+4,0
1910	ℂ	+**100**	**+2,2**	**+0,8**
1911	ℂ	÷ 31	+2,4	÷1,4
1912	ℂ	+ **90**	**+0,4**	**+2,4**
1913	⊙	÷ 89	÷1,9	+0,3
1914	⊙	÷ 49	+4,4	÷0,6
1915	⊙	÷ 54	÷1,6	+2,5
1916	ℂ	+ **70**	÷3,0	**+3,6**
1917	ℂ	÷ 13	+4,7	÷0,4
1918	⊙	÷ 19	÷1,9	+1,7
1919	ℂ	÷ 16	÷3,3	+2,9
1920	⊙	÷ 34	÷0,4	+2,1
1921	⊙	÷ 87	÷0,8	÷0,6

⊕ Great damage in large sections of the country.
⊙ Sporadic appearance without importance.
ℂ Very seldom found.

The years 1889, '94, '95, '96, '97 and 1901 show many severe attacks while the intervening years show few, unimportant attacks, and the years subsequent to 1901 no severe attack whatsoever.

It is certain that the summer climatic conditions, (June, July and August), as well as the climatic conditions of March and April, which determine an early or late sowing and a consequent early or late maturity of the cereals, are important factors in the spread, germination and infection of the rust spores. Late sowing, copious precipitation, low temperature and much cloudiness during the summer cause late maturity and consequent good conditions for a severe attack. Table 1 shows the climatic conditions of June, July and August; the figures for the separate months are added. From this we see that years, during which there were severe attacks of *Puccinia graminis*, contain one or many points of similarity.

Precipitation: above normal in 1889, '94, '95, '97 and 1901.
below " " 1896.
Temperature: above normal in 1889, '94, '96, '97 and 1901.
below " " 1895.
Cloudiness: above normal in 1894, '95, '96 and '97.
below " " 1889 and 1901.

1889—Early sowing; drought in the early summer; the subsequent copious precipitation caused uneven ripening; very late harvest.
1894—Early sowing; late harvest due to the climatic conditions of the summer.
1895—Rather early sowing; harvest slightly later than normal.
1896—Late sowing; early harvest.
1897—Late sowing in unsuitable soil; harvest somewhat later than normal.
1901—Very late sowing; severe attacks of frit-fly (*Oscinis frit*); harvest at normal time.

Either early sowing, unfortunate climatic conditions during ripening and late harvest, or late sowing, often in unsuitable soil, unfortunate climatic conditions during the early part of the summer, but harvest at the normal time, afford good conditions for attacks of black stem-rust.

Conditions similar to those mentioned above characterized the years 1903, '05, '10, '12 and '16.

Precipitation: above normal in 1903, '05, '10, '12 and '16.
Temperature: above normal in 1905, '10 and '12.
below " " 1903 and '16.
Cloudiness: above normal in 1903, '05, '10, '12 and '16.

1903—Early sowing; unfavorable harvest weather, late harvest.

1905—Late sowing; very severe attack of frit-fly; copious precipitation in August; late harvest.

1910—Early sowing; severe drought in the early summer; harvest at normal time.

1912—Late sowing; copious precipitation; much of the crop lodged; late harvest.

1916—Early sowing; germination in unsuitable soil; cold damp summer; late harvest.

On the whole these 5 years seem to reveal similar conditions, for the attacks of black stem-rust, to those in the 6 years referred to above during which very severe attacks of the disease were noted. Nevertheless no severe attacks of black stem-rust were observed during the period 1902–'21. The only possible reason for this lies in the absence of sources of contagion, and a careful 38 years' search of the country shows only one main source—the barberry bush—now practically eradicated. The few stray bushes hidden away here and there are of no importance in this connection; even though, wherever found they should be rooted out without mercy.

It is impossible to reach these results by rational experiments in a country where we may safely say that every spot is infected with black stem-rust; however, if we may consider all of Denmark as a single experimental plot, we have sufficient proof that the eradication of the barberry bush is synonymous with exemption from the destructive attacks of black stem-rust.

LYNGBY, DENMARK.

ELIAS JUDAH DURAND

R. B. Harvey

With Plate XVIII

Dr. Elias J. Durand was born at Canadaigua, New York, March 20, 1870, and died at St. Paul, Minnesota, October 29, 1922. He received the degree of A. B. (1893) and of Doctor of Science (1895) from Cornell University. As Fellow, Assistant and Instructor in Botany he served his Alma Mater from 1893 to 1910. In the latter year he went to the University of Missouri, where he remained as Associate Professor of Botany until 1918. From 1918 to the time of his death he was Professor of Botany at the University of Minnesota.

While Dr. Durand's contributions to botanical science have covered a wide range of subjects, he is best known to his colleagues in botany, both in this country and abroad, for his work in the field of mycology and especially as an authority on the Discomycetes.

Dr. Durand's herbarium has been deposited in the mycological collections of Cornell University, Ithaca, N. Y. It contains 12,087 specimens of Discomysetes fully indexed and classified with indexed notes on all of the specimens. There are also in the collection 6000 microscopic slides prepared from specimens and arranged to facilitate comparison of microscopic characters of the herbarium specimens.

Dr. Durand's publications, while principally in the field of mycology, have included articles on the Bryophyta and a Laboratory Outline of General Botany which has run thru five editions.

In his death, botanical science has lost a faithful scientist, an inspiring teacher, and a kindly personality.

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DEPARTMENT OF BOTANY,
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PHYTOPATHOLOGICAL NOTES

Plant disease survey of England and Wales: This series of publications was briefly mentioned over a year ago. (Phytopathology **11**: 376, 1921). The report for the years 1920 and 1921 is just issued as Miscellaneous Publication No. 38, of the Ministry of Agriculture and Fisheries, 10, Whitehall Place, London.

The two years were as different in climate as any two seasons ever are, hence the report is of especial interest. Three hundred and ninety one diseases are listed with notes on occurrences, severity of attack, relation to weather, etc. Weather charts accompany the report. This is the fourth report to be issued, the series covering the years 1917 to 1921, inclusive.—PERLEY SPAULDING.

Personals. Dr. James R. Weir, who for the past ten years has been in charge of the field investigations of the Office of Forest Pathology, Bureau of Plant Industry, for District 1 of the U. S. Forest Service, (Montana, Idaho, Western Washington, Western Oregon, South Dakota, Minnesota, and Michigan) with headquarters at Missoula and Spokane, has given up this position and is now directing the work of Pathological Collections in the same Bureau. This Office deals with the classification of fungi and maintaining the extensive mycological collections of the U. S. Department of Agriculture. In the immediate future Dr. Weir will accompany the Bureau expedition to the headwaters of the Amazon and other South American regions, as pathologist in rubber investigations. The expedition sailed from New York in July.

Dr. H. H. York, Pathologist in the Office of Forest Pathology, Bureau of Plant Industry, U. S. Dept. Agr., has accepted the position of Forest Pathologist, Division of Forest and Lands, Conservation Commission, Albany, N. Y., beginning July 1, 1923. For the present he continues work upon the white pine blister rust but other forest disease work will be taken up later.

A special institute for phytopathology has been founded at the Landwirtschaftliche Hochschule Bonn-Poppelsdorf, and Dr. E. Schaffnit has been made professor in charge.

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